








**Multiwell test apparatus.**

**Patent number:** EP0596482  
**Publication date:** 1994-05-11  
**Inventor:** LEE CAROLYN (US); RISING DONALD (US)  
**Applicant:** MILLIPORE CORP (US)  
**Classification:**  
- international: B01D61/28; B01D61/18; B01L3/00  
- european: B01D61/18, B01D61/28, B01D61/30, B01L3/00C6D2  
**Application number:** EP19930117839 19931103  
**Priority number(s):** US19920971498 19921104

**Also published as:**

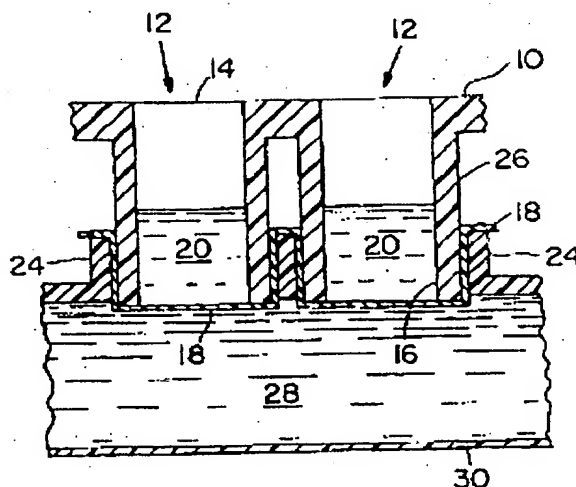
 US5326533 (A1)  
 JP6238176 (A)  
 EP0596482 (B1)

**Cited documents:**

 US4450076  
 WO8607606  
 DE9105550U  
 EP0170098  
 EP0131934  
more >>

**Abstract of EP0596482**

A dialysis apparatus is provided including a multiwell plate (10), a separation membrane (18) and a template (24) having holes which mate with the wells (12). The membrane (18) is sealed to one end of the wells (12) by positioning the template (24) about the outer peripheral surfaces (26) of the wells (12) with the membrane being frictionally held therebetween.

*Fig. 1*

Data supplied from the esp@cenet database - Worldwide

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

**EP 0 596 482 B1**

(12)

**EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention  
of the grant of the patent:  
26.06.1996 Bulletin 1996/26

(51) Int. Cl.<sup>6</sup>: **B01D 61/28**, **B01D 61/18**,  
**B01L 3/00**

(21) Application number: **93117839.6**

(22) Date of filing: **03.11.1993**

(54) **Multiwell test apparatus**

Mehrfachlochtestvorrichtung

Appareil d'essais à cavités multiples

(84) Designated Contracting States:  
**DE FR GB**

(30) Priority: **04.11.1992 US 971498**

(43) Date of publication of application:  
**11.05.1994 Bulletin 1994/19**

(73) Proprietor: **MILLIPORE CORPORATION**  
**Bedford Massachusetts 01730 (US)**

(72) Inventors:  
• **Lee, Carolyn**  
**Sudbury, MA 01776 (US)**  
• **Rising, Donald**  
**Stow, MA 01775 (US)**

(74) Representative: **Henkel, Feller, Hänzel & Partner**  
**Möhlstrasse 37**  
**81675 München (DE)**

(56) References cited:  
**EP-A- 0 131 934** **EP-A- 0 170 098**  
**WO-A-86/07606** **DE-U- 9 105 550**  
**GB-A- 1 595 079** **US-A- 4 450 076**  
**US-A- 5 116 496**

Remarks:

The file contains technical information submitted  
after the application was filed and not included in this  
specification

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

**EP 0 596 482 B1**

## Description

BACKGROUND OF THIS INVENTION

This invention relates to laboratory apparatus useful in the assay of biological and biochemical reactants and is particularly concerned with multiwell apparatus capable of performing a separation process such as ultrafiltration, dialysis or reverse osmosis on a sample.

Ultrafiltration, dialysis and reverse osmosis processes all utilize a membrane to effect separation of component parts of a sample. In dialysis, for example, a sample is processed or purified by placing the sample against one surface of a semipermeable membrane and a pure solvent or solvent/solute dialysate mixture against the opposing surface of the membrane. The molecular weight cut-off (MWCO) of the membrane is selected to retain the sample, yet allow solutes having a smaller molecular size to pass through the membrane. The small molecular size solutes then diffuse throughout the membrane, driven by the concentration gradient of the small molecular size solute across the membrane. Separation of solutes in the sample is achieved by this mechanism.

In practice, a considerable part of the difficulty in performing dialysis lies in isolating the surface in contact with the sample from the opposing surface of the membrane. Cellulose acetate and regenerated cellulose dialysis membranes are notoriously difficult to seal thermally or with adhesives. It is present common practice to tie knots in tubings formed of these polymeric compositions or to utilize pinch clips in order to contain the sample.

Others, such as in DE-U-91 05 550.4, clamp a flat membrane sheet between rigid plates, each containing individual receptacles. Samples are placed in the receptacles of the top plate and dialysate is circulated past the bottom plate. O-rings are utilized to seal receptacles in the top plate from one another. When utilizing this apparatus, it is difficult to attain uniform and effective pressure at the individual O-rings since pressure is not directly exerted on the individual O-rings. This lack of effective pressure application can cause undesirable across-talk between the sample receptacles. Also, since the bottom plate must press the membrane against the entire perimeter of each O-ring, the bottom receptacles form pockets dead-ended against the membrane. Air often is trapped in these pockets and prevents solvent from contacting the membrane. Even when solvent fills each pocket, dialysis is impeded because the dialysate in the pockets does not mix well with the bulk solvent.

Accordingly, it would be desirable to provide an apparatus for simultaneously dialyzing a plurality of samples while avoiding contamination between samples. In addition, it would be desirable to provide such an apparatus which assures direct contact of a separations membrane with a liquid such as a dialysate.

SUMMARY OF THE INVENTION

This invention provides a multiwell apparatus, as disclosed in claim 1, suitable for separating microliter quantities of sample. I.e. the apparatus includes a plate having a plurality of wells, open at one end and having a membrane secured across and sealed to a second end. The membrane is held in place by means of a template having a plurality of holes corresponding to the number and arrangement of the wells which registers with the wells of the plate. The holes of the template are of a size such that when it is pressed about the outside surfaces of the wells in contact with the membrane, the membrane is tightly press fit across all of the second ends of the wells. The template extends a distance along the outside surfaces of the wells past the second end of the wells so that the second end of the well is positioned flush with or away from the bottom surface of the template. In this protruded form, air blockage between the membrane surface exposed to the dialysate and the dialysate in an adjacent container is prevented. Further favourable developments of this multiwell apparatus are disclosed in the respective subclaims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a cross sectional view of the apparatus of this invention.

Fig. 2 is a bottom view of the apparatus of this invention.

Fig. 3 is an exploded view of the apparatus of this invention.

Fig. 4 is a cross - sectional view of an apparatus of this invention which includes a shelf in the well.

DESCRIPTION OF SPECIFIC EMBODIMENTS

This invention provides an improved multiwell apparatus which permits separating a plurality of samples simultaneously while avoiding contamination among samples. A membrane such as a dialysis membrane, an ultrafiltration membrane or a reverse osmosis membrane is sealed to open ends of a multiwell apparatus by means of a template which secures the membrane tightly to one end of each well. The membrane on each well is positioned relative to the template so that the formation of air pockets at the membrane surface is avoided during sample separations such as dialysis.

Referring to Figs. 1 through 3, plate 10 includes a plurality of wells 12. Each well 12 has an open end 14 and a second end 16 which is sealed by membrane 18. Sample 20 is retained within well 12. Membrane 18 is retained in sealing relationship with end 16 by means of template 24. Template 24 has a plurality of holes of a size and position so that they mate with the extension surface 26 of wells 12 adjacent end 16 with the membrane 18 frictionally held between the well surface 26 and the template. The portion of the membrane 18 which contacts end 16 is spaced apart from template 24 so that it

extends into a solution 28 such as a dialysate within container 30 so that air bubbles are avoided between the membrane 18 and solution 28.

Exemplary membranes for use in dialysis for example are formed of a cellulosic such as cellulose, cellulose acetate or cellulose nitrate.

Referring to Fig. 4, the wells 12 of this invention can include a shelf 32 at the end thereof in contact with the membrane 18. The shelf 32 is useful to position a pipette 34 for introducing or removing liquid into or from the well 26 while avoiding contact of the membrane 18 with the pipette 34. The shelf 32 materially reduces the possibility of rupturing membrane 18 with pipette 34.

#### Claims

1. A multiwell apparatus for separating liquid samples with a plate (10) having a plurality of wells (12), each of said wells having a first open end (14), a second end (16) and an outside peripheral surface (26), a single separation membrane (18) in sealing relationship with each of said second ends and a template (24) having a plurality of holes corresponding to the number, the size and the arrangement of the wells (12) which registers with the wells (12) of the plate (10), characterised in that said holes are of a size such that when the template (24) is pressed about the peripheral surface (26) of the wells (12) in contact with the membrane (18), the membrane is tightly press fit across each of the second ends (16), and said template (24) is positioned so as to retain the membrane (18) in sealing relationship with each of the second ends (16) and outside peripheral surfaces (26) of the wells, and so that the template extends a distance along the outside peripheral surfaces of the wells (12) past the second end (16) of the wells so that the second ends are positioned flush with or away from the bottom surface of the template (24).
2. The apparatus of claim 1 including a container (30) for a liquid composition in direct contact with said membrane (18) sealed to the second end (16) of said at least one well.
3. The apparatus of claim 1 including a shelf (32) extending from an inner peripheral surface at said second end (16).
4. The apparatus of claim 1, 2 or 3, wherein said membrane (18) is a cellulosic.

#### Patentansprüche

1. Mehrlochstestvorrichtung zum Trennen von Flüssigkeitsproben, mit einer Platte (10) mit einer Vielzahl von Vertiefungen oder Rohrstutzen (12), von denen jede(r) ein

erstes offenes Ende (14), ein zweites Ende (16) und eine Außenumfangsfläche (26) aufweist,

einer in Abdichtbeziehung zu jedem der zweiten Enden angeordneten, einzigen Trennmembran (18) und einer Schablone (24) mit einer Vielzahl von Bohrungen, die in Zahl, Größe und Anordnung den Rohrstutzen (12) entsprechen und die mit den Rohrstutzen (12) der Platte (10) fluchten bzw. übereinstimmen,

dadurch gekennzeichnet, daß

die Bohrungen eine solche Größe besitzen, daß dann, wenn die Schablone (24) in Berührung mit der Membran (18) um die Umfangsfläche(n) (26) der Rohrstutzen (12) aufgepreßt ist, die Membran mit dichter Anpreßanlage über jedes der zweiten Enden (16) gespannt ist, und

die Schablone (24) so positioniert ist, daß sie die Membran (18) in Abdichtbeziehung zu jedem der zweiten Enden (16) und zu den Außenumfangsflächen (26) der Rohrstutzen hält, und so (positioniert ist), daß sich die Schablone über eine Strecke längs der Außenumfangsflächen der Rohrstutzen (12) über die zweiten Enden (16) der Rohrstutzen hinaus erstreckt, derart, daß die zweiten Enden mit der Unterseite der Schablone (24) bündig abschließen oder von ihr beabstandet sind.

2. Vorrichtung nach Anspruch 1, mit einem Behälter (30) für eine flüssige Zusammensetzung in unmittelbarer Berührung mit der Membran (18), die gegen das zweite Ende (16) des mindestens einen Rohrstutzens abgedichtet ist.
3. Vorrichtung nach Anspruch 1, mit einer von einer Innenumfangsfläche am zweiten Ende (16) abgehenden Auflage oder Leiste (32).
4. Vorrichtung nach Anspruch 1, 2 oder 3, wobei die Membran (18) eine solche aus einer Cellulosesubstanz ist.

#### Revendications

1. Un appareil à cavités multiples pour séparer des échantillons liquides, comportant une plaque (10) présentant une pluralité de cavités (12), chacune desdites cavités présentant une première extrémité ouverte (14), une seconde extrémité (16) et une surface périphérique extérieure (26), une membrane unique de séparation (18) en relation d'étanchéité avec chacune desdites secondes extrémités et avec un gabarit (24) présentant une pluralité d'orifices correspondant au nombre, à la taille et à l'agencement des cavités (12), qui coïncide avec les cavités (12) de la plaque (10), caractérisé en ce que lesdits orifices sont d'une taille telle que, lorsque le gabarit (24) est pressé autour de la surface

périphérique (26) des cavités (12) en contact avec la membrane (18), la membrane est adaptée à force de manière étanche sur chacune desdites secondes extrémités (16), et

ledit gabarit (24) est placé de manière à retenir la membrane (18) en relation d'étanchéité avec chacune desdites secondes extrémités (16) et avec les surfaces périphériques extérieures (26) des cavités, et de telle manière que le gabarit s'étende sur une certaine distance sur les surfaces périphériques extérieures des cavités (12) au-delà de la seconde extrémité (16) des cavités, de telle manière que les secondes extrémités sont situées de niveau avec la surface inférieure du gabarit (24) ou éloignées de celle-ci.

2. L'appareil de la revendication 1, comportant un récipient (30) pour une composition liquide en contact direct avec ladite membrane (18) assujettie de manière étanche à la seconde extrémité (16) de ladite cavité.
3. L'appareil de la revendication 1, comportant un plateau (32) s'étendant à partir d'une surface périphérique intérieure à ladite seconde extrémité (16).
4. L'appareil de la revendication 1,2 ou 3, dans lequel ladite membrane (18) est en un dérivé de cellulose.

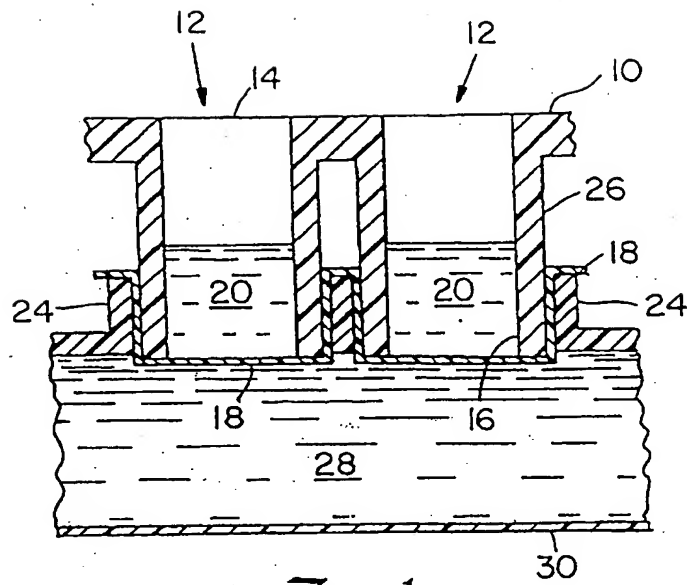


Fig. 1

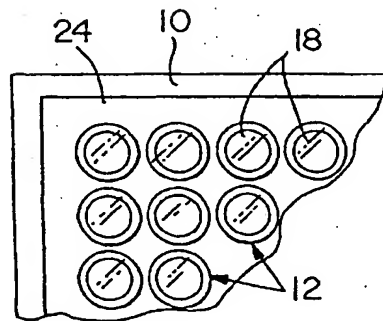


Fig. 2

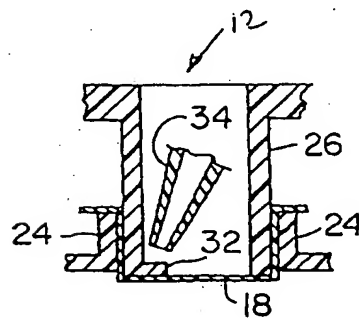


Fig. 4

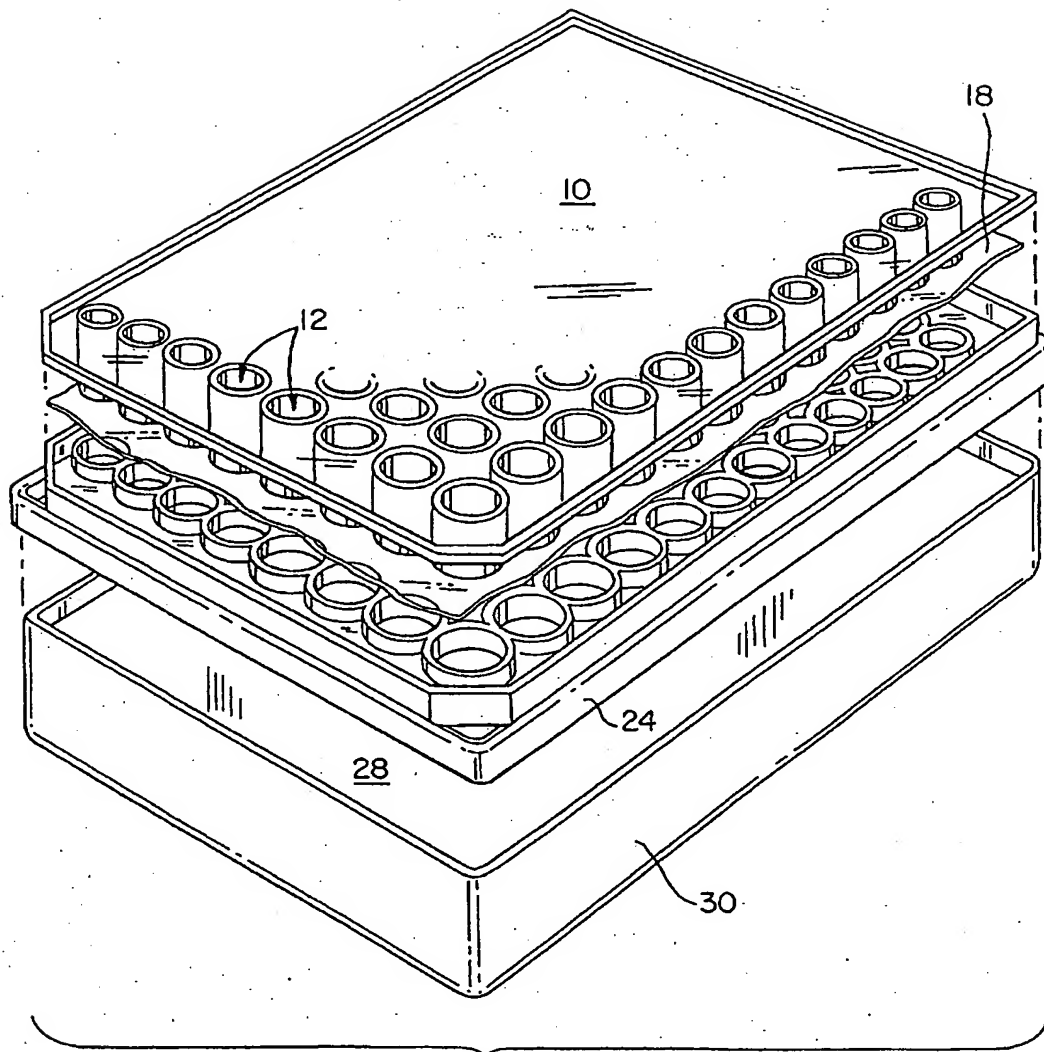


Fig. 3